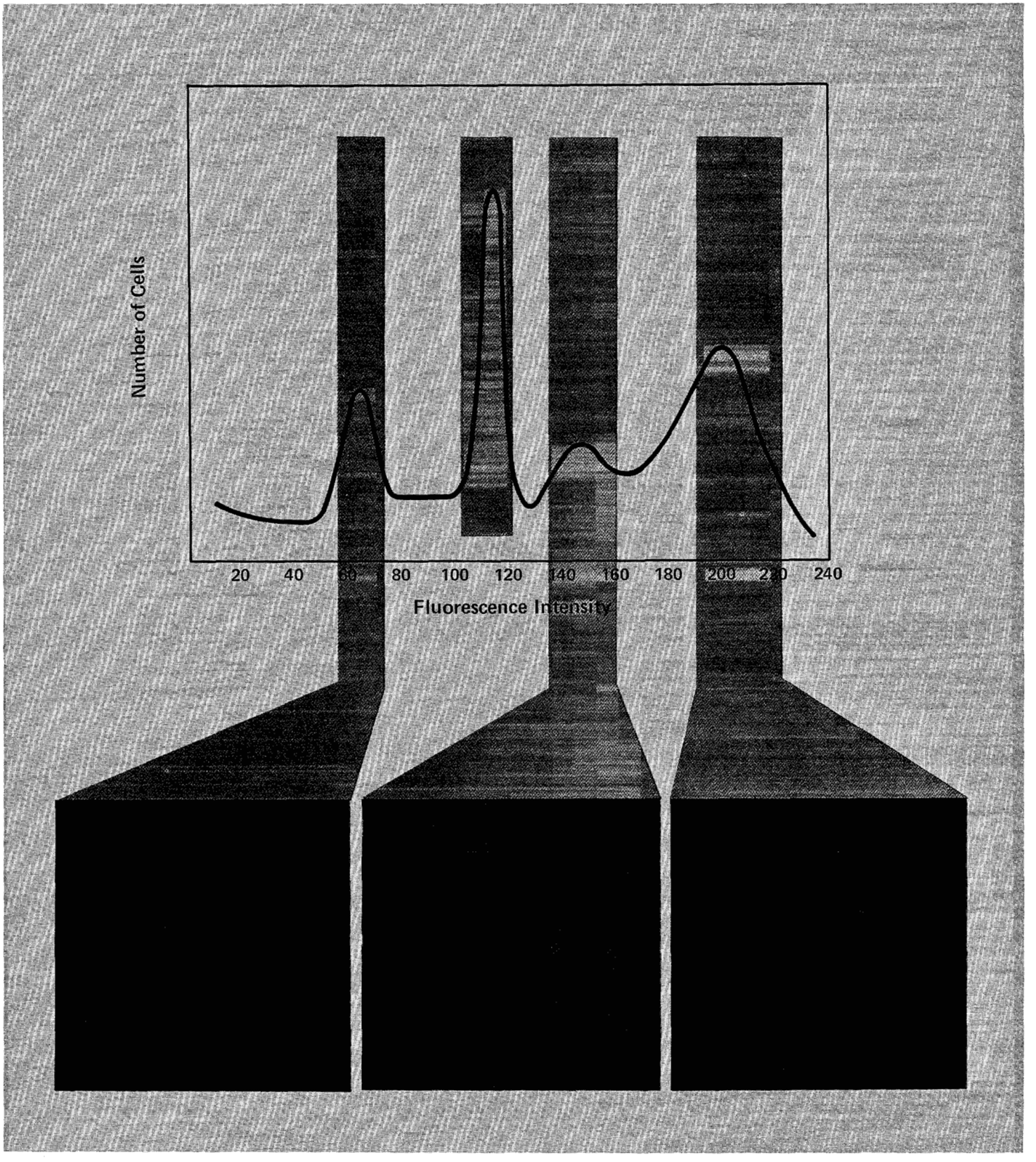

Fig. 13. Intensity distribution of three cell types artificially mixed before fixation in 70% ethanol and stained using a nonspecific propidium iodide procedure. Fluorescence analysis was made using 488-nm laser illumination. Photomicrographs of cells sorted from each of the peaks are shown in the lower part of the figure. Based on cell morphology the cells from the indicated sorting windows were identified as: sort region 1, human white blood cells, magnification of 438 X; sort region 2, cells derived from a methylcolanthrene-induced skin tumor of mice (not shown); sort region 3, methylcolanthrene tumor cells with a few contaminating squamous cells from region 4, magnification of 276 X; region 4, normal human buccal cells (squamous cells) obtained from the lining of the oral cavity, magnification of 276 X. (Figure courtesy of Z. Svitra and John Steinkamp, LASL.)

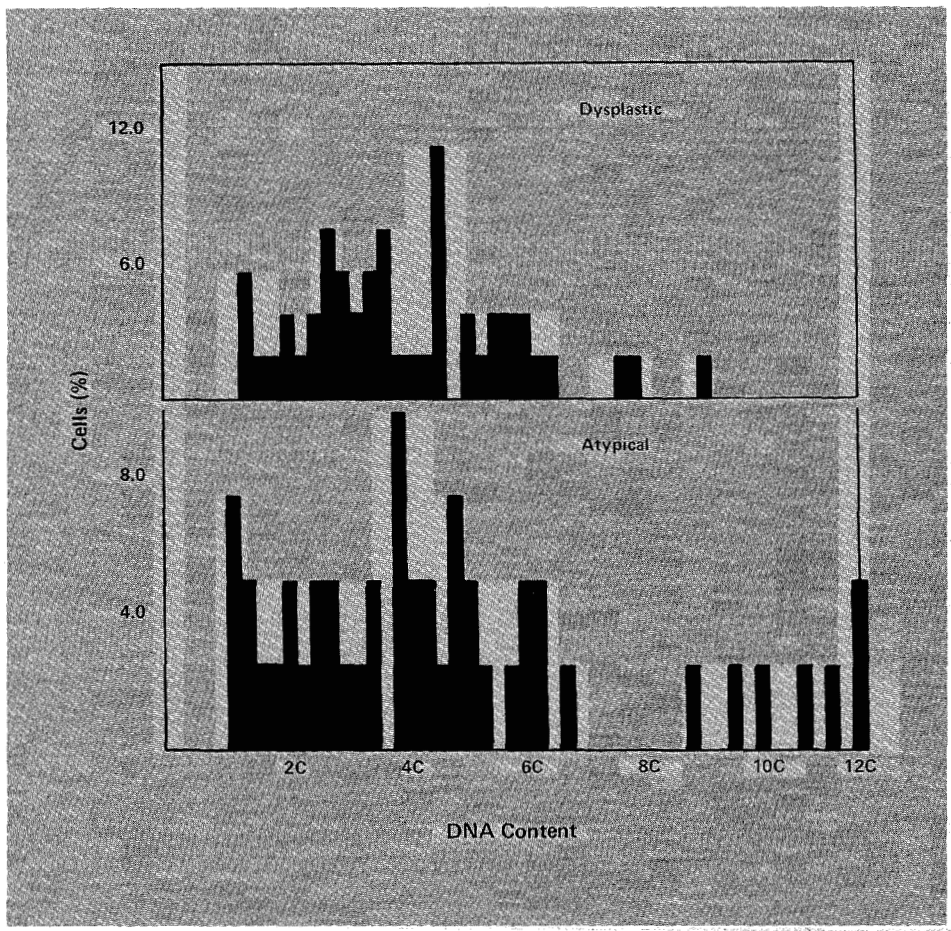

Fig. 14. DNA distributions obtained from abnormal clinical cervical samples —(A) Dysplasia, a recognized precancerous, but temporarily benign condition, and (B) frank cancer (carcinoma in situ). Both conditions have a similar characteristic, that is, cell proliferation as indicated by DNA densities at 4c, 6c, 8c, etc. Resting or mature cells display a DNA value of 2c.

has been used in attempts at cancer diagnosis and in monitoring the effects of chemotherapy on malignant cells. In one application, the cell sorter unscrambled a complex mixture of malignant cells and two types of normal cells as illustrated in Fig. 13. In this instance, the fluorescent staining procedure was only partially specific for the DNA of each cell type. Although the procedure is empirical, it produced clear resolution of the three cell types in the intensity distribution. If a stoichiometric staining procedure had been used, this resolution would not have been achieved. To verify which cells constitute each peak, several thousand cells were sorted from each peak; the results are illustrated in Fig. 13.

Attempts to Automate Cancer Diagnosis

To the cytopathologist, malignant cells have certain distinguishing features that can be observed through the microscope. Because some of these features can be measured with a flow cytometer, we can hope that this instrument may one day be used as an inexpensive automated method for mass screening for cancer. As an example, consider the case of cervical cancer. This disease represents a major worldwide public health concern. In the United States alone, there are approximately 90 million women in the "at risk" age group. Moreover, clear evidence based on case histories tells us that early and accurate detection is the key to a good prognosis. For women who are screened regularly with the "Pap test," survival from this disease is greater than 95% if the disease is detected. However in the United States, mainly for socioeconomic reasons, the majority of the female population never undergoes this screening. Thus for the general, infrequently screened population, survival is more like 50% if the disease is detected. For these reasons, cervical cancer detection has long been a prime candidate for automation.

Under a microscope, the stained nucleus of a malignant cell looks irregular compared to the nucleus of a normal cell. Frequently the nucleus is larger than normal, and it may be less symmetrical. Sometimes the cell too is larger and more irregular and may have a granular appearance. These qualitative differences between normal and malignant cells are fairly easily detected by a trained eye.

The question is, can an instrument do as well? Detailed pattern recognition is slow and expensive, but a flow cytometer can measure several relevant parameters with good accuracy. These are DNA content, nuclear diameter, protein content, and cytoplasmic diameter. On the average these parameters change from normal to malignant cell populations as shown in Table I. The size features (nuclear and cytoplasmic diameters) overlap considerably between normal and malignant cell types. DNA content may increase significantly in malignant cells and the ratio of nuclear diameter to cytoplasmic diameter also tends to increase. The DNA distribution for cells from a frank cervical tumor is shown in Fig. 14. These cells can have DNA

TABLE I

Morphological Characteristics of Normal and Malignant Cells

Feature	Noncycling Cells	Malignant Cells
DNA (G_1)	2c	>2c
Nuclear diameter	<12 microns	>12 microns
Nuc/Cyto diameter	<0.5	>0.5
Cytoplasmic diameter	10-80 microns	20-40 microns

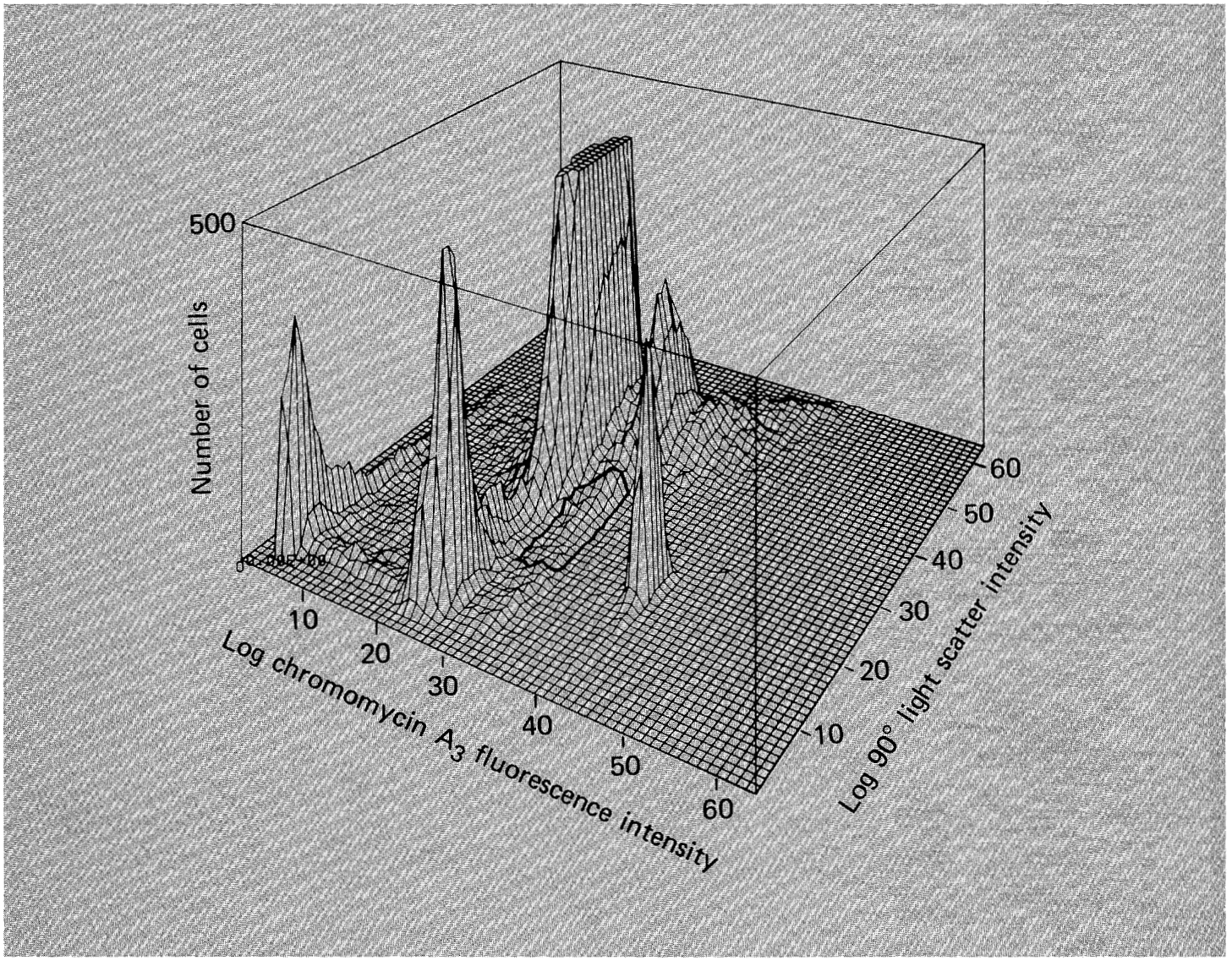

Fig. 15. A two-parameter histogram of cervical and vaginal cells measured by flow cytometry. The histogram is generated by determining chromomycin A3 fluorescence intensity as a measure of cellular DNA content (abscissa in the plane) and 90-degree light-scatter intensity as a measure of cell size (ordinate in the plane) simultaneously on each cell. Thus the location on the plane shows DNA content and cell size, while the height above the plane shows the relative frequency of occurrence. The intensities of the fluorescence and scatter signals are measured and presented logarithmically. An increase in seven channels in either direction corresponds to a doubling of intensity. With the cell sorter, it is possible to identify the cell types that give rise to histogram features. The peak and shoulder at the far left corner of the histogram (the lowest values of fluorescence and light-scatter) are due to cell debris and bacteria. The sharp peak in the foreground with a higher fluorescence intensity and low light-scatter is due to white blood cells; the large peak at equal fluorescence intensity but higher scatter values is due to intermediate and superficial epithelial cells. The small shoulders to the right of the white cell and epithelial cell peaks are due to cell aggregates. The sharp peak at even higher fluorescence is due to fluorescent microspheres that were added for machine calibration. The identities of the cells responsible for each feature have been confirmed by cell sorting and morphological analysis. (Figure courtesy of R. Jensen, Lawrence Livermore Laboratory)

values as high as 12c, or 6 times the normal noncycling value. Since the geometric features have been shown clinically to have diagnostic value, sets of parameters such as DNA and size have been investigated for use in automated diagnosis of cervical cancer by flow cytometry.

At the Lawrence Livermore Laboratory, clinical material has been examined for its DNA content and cell size. The DNA was measured by fluorescence, and the cell size was determined from light-scattering measurements. The data result in three-dimensional histograms of the two variables, as shown in Fig. 15 from an abnormal specimen.

Malignant and premalignant cells contain elevated amounts of DNA and tend to be intermediate in size. A large fraction of the abnormal cells in Fig. 15 show fluorescence and light-scatter signals that are localized to the right of the main peaks (in the heavily outlined area). A 20- to 30-fold enrichment of abnormal cells can be obtained for